APS DIALOG Search 9/16/98 VSP

(FILE 'USPAT' ENTERED AT 07:34:29 ON 26 AUG 1998)
L1 199 S 0157
L2 80 S 0157(W)H7 OR O157:H7 OR O157?
L3 277 S 0157?
L4 300 S L1-L3
L5 9 S L4 (3P) VACCIN?
L6 21 S L4 AND COLI AND VACCIN?
L7 13 S L6 NOT L5

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9 ANSWERS ARE AVAILABLE. SPECIFIED ANSWER NUMBER EXCEEDS ANSWER SET SIZE
ENTER ANSWER NUMBER OR RANGE (1):1-9

1. 5,798,260, Aug. 25, 1998, Escherichia coli O157:H7 epithelial adhesin; Phillip I. Tarr, et al., 435/252.3, 320.1; 536/23.7 [IMAGE AVAILABLE]

US PAT NO: 5,798,260 [IMAGE AVAILABLE] L5: 1 of 9

ABSTRACT:

A continuous segment of chromosomal DNA from E. coli O157:H7, isolated on plasmid pSC(overlap) (ATCC No. 69648), encodes an adhesin (SEQ ID NO:4) that mediates bacterial colonization of bovine intestines.

2. 5,747,293, May 5, 1998, Intimin-like proteins of E. coli; Gordon Dougan, et al., 530/402, 350, 825 [IMAGE AVAILABLE]

US PAT NO: 5,747,293 [IMAGE AVAILABLE] L5: 2 of 9

ABSTRACT:
The invention is related to antibodies, particularly monoclonal antibodies, which recognize particularly epitopes of the intimin protein of enteropathogenic E. coli and enterohemorrhagic E. coli, methods of detecting such E. coli by use of these antibodies, and kits containing these antibodies for diagnosis.

3. 5,730,989, Mar. 24, 1998, Oral vaccine against gram negative bacterial infection; D. Craig Wright, 424/241.1, 197.11, 249.1, 255.1, 258.1, 261.1, 450 [IMAGE AVAILABLE]

US PAT NO: 5,730,989 [IMAGE AVAILABLE] L5: 3 of 9

ABSTRACT:
The present invention concerns an oral preparation useful as an immunizing agent or vaccine against gram negative bacterial infection. This oral preparation can also be used as a treatment for those infected with gam negative bacteria. The preparations can be used against any gram negative bacterial infection, including Escherichia coli, Shigella flexneri 2a, and Salmonella enteriditis.

4. 5,654,417, Aug. 5, 1997, Nucleic acid probes for detecting E. coli O157:H7; Phillip I. Tarr, et al., 536/24.32; 435/6; 536/23.1, 24.3 [IMAGE AVAILABLE]

APS DIALOG search 9/98

(FILE 'USPAT' ENTERED AT 10:45:01 ON 18 SEP 1998)
E INTIMAIN
E INTIMIN
L1 3 S E3 OR E7
E INVASIN
L2 17 S E3-E5
L3 18 S L1 OR L2
L4 9 S L3 (2P) (ANTIBOD? OR POLYCLONAL? OR MONOCLONAL OR MONO-C
LON

=> d hit 1-9

Nystrom EA.

US PAT NO: 5,800,821 [IMAGE AVAILABLE] L4: 1 of 9

DRAWING DESC:

DRWD(2)

FIG. 1 is a graph showing SLT-I B subunit expression in B. subtilis using the SPAC promoter (pDA1) or glutamine synthetase promoter (pDA6) with and without added glutamine in the culture medium (L. broth). The B subunit in culture supernatants was measured by ELISA using a B subunit specific **monoclonal antibody** as the capture molecule, and is given as absorbance units. Cultures reached stationary phase at 4-5 hours.

DRAWING DESC:

DRWD(3)

FIG. 2 is a blot showing a HEp-2 cell binding assay in parental (negative control) E. coli (Lane A), E. coli expressing DppE-Inv192 fusion protein (Lane B) and E. coli expressing the whole **Invasin** molecule (positive control) in Lane C.

DRAWING DESC:

DRWD(4)

FIG. 3 is a bar graph showing results when whole cells or spheroplasts were incubated with iodinated **monoclonal antibody** 3A2-1 directed toward the C-terminus of **Invasin**. Bound counts were determined following washing.

DRAWING DESC:

DRWD(5)

FIG. 4 is a Western blot of DppE-Inv192 expression in B. subtilis parental cells (lane B), recombinant spheroplast cytoplasm (lane C), recombinant spheroplast membranes (lane D), whole recombinant spheroplasts (lane E), and whole recombinant cells (lane F). **Invasin** expression in E. coli (positive control) is shown in lane A.

DRAWING DESC:

DRWD(6)

FIG. 5 is a graph showing whole cells from E. coli (with or without a plasmid coding for **Invasin**) or B. subtilis HJS31 (Inv -) and recombinant B. subtilis (CwbA-Inv192 +) incubated with iodinated 3A2-1. The total counts bound following washes are shown.

DRAWING DESC:

DRWD(7)

FIG. 6 is a graph showing binding of murine **antibodies** to **Invasin** in an ELISA from 15 mice orally immunized with spores whose vegetative cells expressed CwbA-Inv fusion proteins. The results are expressed as absorbance units (405 nm).

DETDESC:

DETD(23)

There are several ways to determine where the expressed fusion proteins are situated in the spore coat or cell wall. One way is to determine if radiolabeled **monoclonal antibody** 3A2-1 (which is directed toward the C-terminal 192 amino acids of **Invasin**) is able to bind preferentially to the spores or vegetative cells (as we found with spheroplasts for the DppE-**Invasin** fusion protein, and for whole cells with the CwbA-Inv192 fusion protein). Another is to use indirect or direct fluorescence with either a FITC conjugated anti-murine **antibody** or fluoresceinating 3A2-1, respectively. It may be necessary to use ultrastructural morphological techniques to determine the site of the fusion protein in some cases, e.g., by using immunogold techniques with 3A2-1.

DETDESC:

DETD(24)

We have expressed proteins, such as Shiga-like toxin I B subunit (SLT-I B) and portions of **Invasin** from Y. pseudotuberculosis, in B. subtilis. We have expressed SLT-I B from both plasmid and chromosomal sites within B. subtilis and clearly demonstrated the expressed protein in culture supernatants of the genetically altered B. subtilis cells. We have constructed genetic fusions between B. subtilis cell membrane or cell wall proteins and the carboxy-terminal domain of **Invasin**, and have expressed the resultant fusion proteins in B. subtilis. Our experiments established that it is possible to target a portion of **Invasin** to either the cell membrane or cell wall of B. subtilis, and that the fusion protein retains the phenotypic characteristics of **Invasin**, in terms of HEp-2 cell binding. Additionally, we have demonstrated that the CwbA-Inv fusion proteins are immunogenic when expressed from vegetative B. subtilis following oral administration of spores encoding the CwbA-Inv fusions.

DETDESC:

DETD(38)

The dppE gene encodes a dipeptide binding protein which is secreted from B. subtilis but remains attached to the membrane by a lipoprotein

linkage. We hoped therefore that dppE would be exposed on the cell surface. The C-terminus of the dppE gene was fused in frame to DNA encoding the 192 C-terminal amino acids of **Invasin** from Yersinia pseudotuberculosis. This **Invasin** protein is a 103 kDa protein (986 amino acids) which has been shown to be localized in the outer membrane (Isberg et al., Cell, 1987, 50:769-78). The integrin binding domain of **Invasin** has been identified as being within the carboxyl-terminal 192 amino acids (Leong et al., EMBO J., 1990, 9:1979-89). Thus, the C-terminal 192 amino acid portion of **Invasin** has the potential to cause bacterial cells on whose outer surface it is expressed to attach to mammalian cells.

DETDESC:

DETD(39)

To construct the DppE-**Invasin** fusion protein, we cloned the whole of the dppE gene, excluding the termination codon, using PCR. The primers were designed to have EcoRI and KpnI sites upstream, and a SacI site downstream to allow cloning into the EcoRI and SacI sites of pBluescript SK and to facilitate sequencing of the fusion gene. The dppE gene was then cloned as an EcoRI-SacI fragment into the EcoRI and SacI sites of pAF1 (Fouet et al., 1990, supra). The glutamine synthetase promoter was cloned upstream of the dppE gene as an EcoRI-KpnI fragment. The nucleotides encoding the carboxy terminal 192 amino acids of **Invasin** were then amplified using PCR. SacI sites were appended to each end to allow in-frame insertion into the SacI site of the previously constructed plasmid. The result was a plasmid (pDA74) containing a fusion gene consisting of the whole of the dppE coding sequence and the portion of the carboxy terminus of **Invasin** which is responsible for binding to cells. The gene fusion was positioned between the amino and carboxy terminal portions of the amyE gene as before, to allow integration into the B. subtilis chromosome.

DETDESC:

DETD(40)

Expression of the DppE-**Invasin** fusion protein was demonstrated in E. coli. The fusion protein (approximately 80 kDa) was detected by Western blot using a **monoclonal antibody** (3A2-1) directed toward the C-terminus of **Invasin** (Leong et al., 1991, supra). We determined the ability of the fusion protein expressed in E. coli to bind HEp-2 cells by transferring the fusion protein onto nitrocellulose and then incubating the membrane with HEp-2 cells. Bound HEp-2 cells were detected by virtue of their endogenous alkaline phosphatase activity. HEp-2 cells bound to **Invasin** present in the positive control E. coli (FIG. 2, Lane C), and to the DppE-Inv192 fusion protein in lane B (FIG. 2). Non-specific binding can be seen in lanes A and B in a band just below the level of the DppE-Inv192 fusion protein (FIG. 2). pDA74 was then linearized and introduced into B. subtilis. The fusion protein was not only expressed in B. subtilis but was also able to bind HEp-2 cells, as determined by blotting.

DETDESC:

DETD(41)

In order to increase expression of the fusion protein, pDA74 was

introduced into B. subtilis strain HJS31 (a glnR mutant) in which the glutamine synthetase promoter is expressed constitutively. The physical location of the fusion protein in the B. subtilis strain carrying the DppE-Inv192 fusion was determined using a variety of techniques. Binding of .sup.125 I-labeled **monoclonal** anti-**Invasin antibody** 3A2-1 to the fusion protein was confirmed by an autoradiograph of a Western blot. When binding to whole bacterial cells was tested, the iodinated **antibody** bound to both the parental and the DppE-Inv192 recombinant strain to approximately the same extent (FIG. 3). However, when binding to spheroplasts (prepared by incubating whole cells with lysozyme, and confirmed by microscopy) was determined, there was approximately 5-fold more binding of the **antibody** to the recombinant spheroplasts than to control spheroplasts (FIG. 3).

DETDESC:

DETD(44)

Example 3: Expression of B. subtilis Cell Wall Protein-**Invasin** Fusion

DETDESC:

DETD(45)

B. subtilis produces a number of cell wall proteins. Two of the best studied classes of cell wall proteins are the autolysins and their associated modifier proteins, which are thought to be important in regulating autolysin activity. Kuroda et al. (J. Bacteriol., 1991, 173:7304-12) cloned and sequenced a major autolysin gene, designated cwlB. Kuroda et al. (1991, supra) subsequently sequenced a gene for a modifier protein, designated cwbA, which is immediately upstream of cwlB. We constructed a genetic fusion using the whole of cwbA and the DNA encoding the C-terminal 192 amino acids of **Invasin**. The cwbA gene encodes a polypeptide of 705 amino acids with a molecular mass of 76,725 Da (Kuroda et al., supra). In order to clone the cwbA gene, we designed PCR primers which annealed to the 5' end of the gene and included the ribosomal binding site. The reverse primer was designed to anneal to the downstream portion of the gene and to exclude the termination codon. The upstream primer contained a KpnI site and the downstream primer a SacI site. Following PCR, we obtained a 2140 bp fragment containing the cwbA gene, as expected. Following appropriate restriction digestion, the fragment was inserted into the KpnI and SacI sites of pBluescript SK. The 5' portion of the insert was sequenced to check that it was the correct gene. The next stage of the construction involved moving the KpnI-SacI fragment from the pBluescript vector into plasmid pDA74 (described above) which placed the cell wall protein gene under the glutamine synthetase promoter. Next, the DNA encoding the terminal 192 amino acids of **Invasin** was amplified using PCR primers with SacI restriction sites on each end. The PCR product was inserted downstream of and in-frame with cwbA. The fusion site was sequenced and the final plasmid containing the cwbA-inv192 gene fusion was designated pDA84. The gene fusion was integrated into the amyE locus of B. subtilis HJS31. This strain produced detectable levels of the fusion protein (by Western blot with **monoclonal antibody** 3A2-1). The size of the reactive band was about 100 kDa, as expected.

DETDESC:

DETD(46)

When B. subtilis expressing the CwbA-Inv192 fusion protein was grown to early stationary phase and the cells treated with lysozyme in the presence of 0.5M sucrose to maintain the spheroplasts, there was clearly more CwbA-**Invasin** protein, as a proportion of the total protein, in the lysozymal supernatant than in the spheroplasts. Thus it appeared that the CwbA-**Invasin** protein was predominantly associated with the cell wall. When we examined the binding of iodinated 3A2-1 to B. subtilis HJS31 and the recombinant CwbA-Inv192 expressing B. subtilis at three different points in their growth curve, there was a clear difference in the binding of the **monoclonal antibody** to the two strains (FIG. 5). There was a similar difference when the same experiment was performed with E. coli which did and did not express **Invasin** (FIG. 5). The same number of bacterial cells was used at each growth point, and the ratio of binding in overnight cultures between **Invasin** positive and negative B. subtilis (8.1) and E. coli (6.0) was similar. There was some suggestion that the binding of 3A2-1 to the Inv(+) Bacillus increased as the cultures grew, since the binding to control HJS31 did not really change during the course of the culture.

DETDESC:

DETD(47)

The binding of iodinated **monoclonal antibody** to spheroplasts was then assessed. Whole cells and spheroplasts of B. subtilis HJS31 and the recombinant B. subtilis, which had been grown to an optical density (600 nm) of approximately 1.0, were incubated with iodinated 3A2-1 and the bound counts compared. As shown in Table 1, the ratio of bound counts between the two strains suggested that removing the cell wall also eliminated most of the preferential binding of 3A2-1.

DETDESC:

DETD(63)

To determine whether or not the otherwise unmodified spores of B. subtilis containing the DNA encoding the CwbA-Inv fusion proteins could induce an immune response in vivo, spores were administered orally, directly into the stomachs, of 19 mice using a gavage needle. Each mouse received four doses of 1.times.10.sup.10 spores at 10 day intervals. Animals were bled prior to the experiment (for control serum) and 10 days after the third and fourth inocula. The presence of IgG serum **antibodies** to **Invasin** was measured using an ELISA format with recombinant **Invasin** as the capture molecule. Five of the mice developed serum **antibodies** to **Invasin** with an increasing titer after multiple immunizations (FIG. 6). The **antibodies** detected by ELISA were also functional and able to neutralize **Invasin**, in that they were able to inhibit invasion by Inv-positive E. coli into mammalian HEp-2 cells.

DETDESC:

DETD(64)

We know that the spores themselves do not express the **Invasin** protein, and therefore the only way these mice could develop an immune response to **Invasin** is if the spores germinate and express the fusion antigen. In these experiments, we did nothing to optimize colonization

and survival of the vector in the gastrointestinal tract. It is possible that prolonged recycling of spores could occur by coprophagy, typical in mice. However, this would not produce a specific **antibody** response without germination and expression of the protein antigen.

DETDESC:

DETD(65)

The data from Examples 1-5 show that the SLT-I B subunit can be expressed in B. subtilis and that this protein is exported from the cell, presumably by virtue of its own signal peptide. These experiments show that it is possible to construct and express two different fusion proteins (using in this case the C-terminus of **Invasin**) directed toward either the cell membrane or the cell wall of Bacillus. The experiments also show that B. subtilis vegetative cells are able to survive in the GI tract of mice at least two days following oral gavage. This indicates survivability of the cells, one of the requirements of our vaccine constructs. We also demonstrate that spores can be recovered to at least 7 days post-gavage, which is clearly longer than the GI transit time, and our data show that following oral immunization with spores, germination can occur within the gastrointestinal tract and the resulting vegetative cells can express the inv fusion gene in sufficient quantities to result in a systemic immune response.

US PAT NO: 5,798,260 [IMAGE AVAILABLE] L4: 2 of 9

SUMMARY:

BSUM(12)

Outer membranes of E. coli O157:H7 competitively inhibit adherence to HEp-2 cells, an inhibition which is not due to H7 flagellin or O157 lipopolysaccharide (65). Adherence of E. coli O157:H7 to HEp-2 cells was reduced, but not abolished, by **antibody** to a 94 kDa outer membrane protein (64). **Antibodies** to enterotoxigenic E. coli colonization factor antigens I and II do not detect surface structures on E. coli O157:H7 (78). E. coli O157:H7 do not have sequences homologous to the EPEC adherence factor plasmid or to the diffuse adherence adhesin (71).

SUMMARY:

BSUM(13)

Some investigators have suggested that the epithelial cell adhesin of E. coli O157:H7 is encoded by its eae gene (17). E. coli O157:H7 eae is related to inv, which encodes Yersinia **invasin**, which also functions as an adhesin, and EPEC eae, which encodes **intimin**. An eae deletion mutant of E. coli O157:H7 neither adhered to HEp-2 cells nor caused the attaching and effacing (AE) lesion in newborn pigs (17). When deletion mutants were complemented in trans by an intact eae gene, the strain could again cause the AE lesion, but still could not adhere in vitro. However, data from other groups suggest that the eae gene product is not an adhesin for E. coli O157:H7. First, despite sequence homology to inv in its bacterial localization and transmembrane domains, the receptor binding domain of E. coli O157:H7 eae is quite dissimilar (4,82). Second, an eae insertional mutant in E. coli O157:H7 retained the ability to adhere to HEp-2 cells in a quantitative adherence assay (41). Third, an eae gene product does not confer adherence on nonadherent laboratory

strains of E. coli. (Jerse, A., et al., Proc. Natl. Acad. Sci. USA 87:7839-7843, 1990) Thus, a molecule other than the eae gene product in E. coli O157:H7 appears to be the primary adhesin of E. coli O157:H7 for bovine epithelial cells, enabling this human pathogen to colonize the bovine gastrointestinal tract.

SUMMARY:

BSUM(14)

Bacterial adhesins, when used as immunogens, prevent disease or colonization of mucosal sure by bacteria in many animals (1,18,21,29,30,36,49,50,55,61,68,81, which are hereby incorporated by reference). The reduction of E. coli O157:H7 at its bovine source would enhance the microbiologic safety of food derived from cattle, and lessen the environmental biohazard risk posed by the approximately 100,000 cattle detectably infected with E. coli O157:H7 at any one time in the United States. The availability of **antibody** for passive immunization would greatly mitigate the harm engendered by outbreaks of this infection.

US PAT NO: 5,759,551 [IMAGE AVAILABLE] L4: 3 of 9

SUMMARY:

BSUM(13)

Moreover, protein linkage to LHRH is problematic because the majority of immune responses are directed to the carrier rather than to LHRH (the mass of the toxin molecule(s) is much greater than that of LHRH). This phenomenon leads to carrier-induced immune suppression. Because the majority of cancer or endometriosis patients have been previously immunized with diphtheria and tetanus vaccines as part of mandatory immunization programs, **antibody** and/or suppressor T cell responses directed to tetanus or diphtheria toxin components of the vaccines can interfere with the subsequent immune responses to toxin-linked LHRH immunogens.

SUMMARY:

BSUM(14)

Accordingly, an immune enhancer that is suitable for human use, inexpensive and capable of stimulating an early and strong immune response to LHRH has been sought. Likewise this immune enhancer should avoid carrier-induced suppression. Hence, it has been found that peptides containing particular structural arrangements of a Th epitope alone or linked to an **invasin** domain (as an immune enhancer) and LHRH (as immunogen) are effective in stimulating the production of **antibodies** against LHRH.

SUMMARY:

BSUM(15)

The present invention relates to peptides, preferably synthetic peptides, which are capable of inducing **antibodies** against LHRH that lead to the suppression of LHRH levels in males or females. The subject peptides are useful for inducing infertility and for treating prostatic

hyperplasia, androgen-dependent carcinoma, prostatic carcinoma, testicular carcinoma, endometriosis, benign uterine tumors, recurrent functional ovarian cysts (severe) premenstrual syndrome or for prevention or treating estrogen-dependent breast cancer. In particular, peptides of this invention have a Th epitope and carboxyl-terminal LHRH, or a peptide analog of LHRH. These peptides are effective as immunogens and therapeutics. The peptides of this invention are capable of reducing serum testosterone to levels comparable to those obtained by orchiectomy (castration) and of causing reversible atrophy of the testes, prostate and other androgen- or estrogen-dependent sex organs. Optionally, the peptides have an **invasin** domain as an immune stimulator.

SUMMARY:

BSUM(17)

A further aspect of the invention relates to a method for suppressing activity of circulating LHRH levels in a mammal by administering one or more of the subject peptides to the mammal for a time and under conditions sufficient to induce functional **antibodies** directed against said LHRH. Suppression of LHRH activity is useful to treat prostatic hyperplasia, androgen-dependent carcinoma, prostatic carcinoma, testicular carcinoma, endometriosis, benign uterine tumors, recurrent functional ovarian cysts or (severe) premenstrual syndrome, or to prevent or treat estrogen-dependent breast cancer. More particularly, the invention provides a method for inducing infertility in a mammal by administering the subject vaccine compositions to the mammal for a time and under conditions to produce an infertile state in the mammal. Similarly, this invention relates to a method for treating androgen-dependent carcinoma by administering the subject vaccine compositions to the mammal for a time and under conditions to effect regression or prevent growth of the carcinoma.

SUMMARY:

BSUM(18)

Yet another aspect of the invention relates to an immunogenic synthetic peptide of about 30 to about 90 amino acids which contains an immunostimulatory **invasin** domain, a helper T cell (Th) epitope and a peptide hapten. These three elements of the peptide can be covalently joined in any order provided that either the immunoreactivity of the peptide hapten is substantially preserved or that immunoreactivity to a self-peptide can be generated. The peptide haptens of the invention include self-peptides LHRH, amylin, gastrin (gastrin.sub.34 and gastrin.sub.17), gastrin releasing peptide and a peptide derived from the CH4 domain of the IgE molecule as well as peptides from Chlamydia trachomitis, human immunodeficiency virus, Plasmodium berghei, or any other B cell epitope (such as from pathogenic organisms) or a CTL (cytotoxic T cell)-generating epitope. Further these peptides have one or more amino terminal (A).sub.n groups, where A is an amino acid, a-NH.sub.2, tripalmitoyl cysteine or a fatty acid and n is from 1 to about 10. The three elements of the subject peptides can be separated by a (B).sub.o spacer group, where B is independently any amino acid and o is from 0 to about 10.

DRAWING DESC:

DRWD(30)

FIG. 30 graphically depicts levels of anti-LHRH specific **antibody** produced in rats following immunization with Inv: HBsAgT.sub.h : LHRH (peptide 32). Peptide 32 consists of a segment of Yersenia adhesion molecule, **Invasin**, linked to a T cell helper epitope derived from the hepatitis B virus surface antigen linked to LHRH. Five sexually mature Sprague-Dawley male rats per group were given peptide 32 equivalent to 100 .mu.g of peptide A by subcutaneous administration. The antigen was formulated on aluminum hydroxide and given at week 0, 3 and 6. The control group was given unmodified LHRH on alum using the same immunization schedule.

DETDESC:

DETD(8)

5. Covalent Addition of an **Invasin** Domain as an Adjuvant. The **invasins** of the pathogenic bacteria Yersinia spp. are outer membrane proteins which mediate entry of the bacteria into mammalian cells (Isberg and Leong, 1990, Cell 60:861). Invasion of cultured mammalian cells by the bacterium was demonstrated to require interaction between the Yersinia **invasin** molecule and several species of the .beta.1 family of integrins present on the cultured cells (Tran Van Nhieu and Isberg, 1991, J. Biol. Chem. 266:24367). Since T lymphocytes are rich in .beta.1 integrins (especially activated immune or memory T cells) the effects of **invasin** upon human T cell have been investigated (Brett et al., 1993, Eur. J. Immunol. 23:1608). It is thought that integrins facilitate the migration of immune T cells out of the blood vessels and through connective tissues to sites of antigenic challenge through their interaction with extracellular matrix proteins including fibronectin, laminin and collagen. The carboxy-terminus of the **invasin** molecule was found to be costimulatory for naive human CD.sup.4 +T cells in the presence of the non-specific mitogen, anti-CD3 **antibody**, causing marked proliferation and expression of cytokines. The specific **invasin** domain which interacts with the .beta.1 integrins to cause this stimulation also was identified (Brett et al., 1993). Because of the demonstrated T cell co-stimulatory properties associated with this domain, it can be linked it to promiscuous T.sub.h epitope: LHRH constructs.

DETDESC:

DETD(10)

7. Selection of an Adjuvant/Emulsion Formulation to Maximize **Antibody** Responses. In addition to the significant adjuvanting properties associated with covalent modifications of the T.sub.h epitope: LHRH constructs (e.g the **invasin** domain and/or Pam.sub.3 Cys), addition of exogenous adjuvant/emulsion formulations which maximize immune responses to the LHRH immunotherapeutic immunogens have been investigated. The adjuvants and carriers that have been evaluated are those: (1) which have been successfully used in Phase I human trials; (2) based upon their lack of reactogenicity in preclinical safety studies, have the potential for approval for use in humans; or (3) have been approved for use in food and companion animals.

DETDESC:

DETD(247)

Further Modification of the LHRH Immunogens to Amplify **Antibody** Induction: Addition of an **Invasin** Domain

DETDESC:

DETD(248)

T cell activation can also be brought about by LHRH that is covalently linked to a specific fragment from the **invasin** protein of the pathogenic bacteria Yersinia spp. Peptide 32, in which a domain of the **invasin** protein is linked to the HBs T.sub.h epitope: LHRH construct (i.e. Inv.sub.718-732 +peptide 18) has been synthesized. Peptide 32 is organized in five linear domains, from the amino- to the carboxyl-terminus, as follows: the **invasin** T cell stimulator (Inv), a glycine spacer (GG), the hepatitis B surface antigen helper T cell epitope (HBsAg T.sub.h 1), a glycine spacer (GG), and LHRH. Peptide 32 is thus represented as: Inv: GG: HBsAg T.sub.h 1: GG: LHRH. The following provides a specific example of the significant efficacy imparted to the LHRH immunogen by the addition of the **invasin** domain. The experimental design is the same as in Example 5 except as indicated otherwise. Experimental Design:

DETDESC:

DETD(256)

FIG. 30 describes the LHRH-specific **antibody** titers produced in rats immunized with peptide 32. Significant titers were achieved after the first booster immunization (at 3 weeks) which continued to increase following the second booster immunization at 6 weeks. By week 8, 4 of 5 animals exhibited LHRH **antibody** titers above 2 nmole/L. Control animals immunized with an Inv.sub.718-732 : LHRH construct, lacking a T.sub.h epitope, did not produce any measurable LHRH-specific **antibody**. Serum testosterone levels (FIG. 31) fell precipitously in the animals responding to peptide 32, and by week 8, testosterone levels were below the threshold for castration. Serum testosterone in these animals remained unmeasurable for the remainder of the experiment. As demonstrated by FIG. 32, dramatic organ atrophy was achieved in the four responding animals. The testes of control animals immunized with peptide 18 (HBs T.sub.h : GG: LHRH; lacking the **invasin** epitope) were unaffected at the end of this experiment (i.e. at week 10). This result is especially important since the **invasin**-containing LHRH peptide was formulated on alum and administered subcutaneously. Previous studies with LHRH linked to high molecular weight carrier molecules, e.g. tetanus and diphtheria toxins, required formulation with Freund's complete adjuvant or other reactogenic adjuvants to achieve any significant degree of efficacy.

DETDESC:

DETD(258)

1. The **invasin** fragment provides a significant improvement in the immune responses to T.sub.h : LHRH constructs.

DETDESC:

DETD(262)

An experiment testing the efficacy of the cocktail of immunogens as described in Examples 10 and 11, was conducted except that the HBs T.sub.h : GG: LHRH construct was replaced with peptide 32. The protocol for this example is identical to that used in Example 11. As above, animals received 100 .mu.g of peptide on alum, administered at 0, 3 & 6 weeks. As demonstrated by FIG. 33, rapid and potent anti-LHRH **antibody** responses were produced in response to immunization with the **Invasin** fragment-containing cocktail when formulated on alum. By 8 weeks, 6 of 6 animals receiving the peptide 32-containing cocktail expressed serum testosterone levels (FIG. 34) below the castration threshold (i.e. less than 0.5 nmole/L). In contrast, 4 of 6 animals receiving an equivalent dose of peptide 32 alone on alum had castration levels of testosterone. These data suggest that any genetic variability associated with responses to the **invasin** fragment are overcome by its presentation in the cocktail containing the different T.sub.h constructs. FIG. 35 describes the testis weights at the end of the experiment (at 10 weeks). Five of 5 animals receiving the peptide 32-containing cocktail of immunogens exhibited significant organ atrophy and by histological examination were functionally sterile.

DETDESC:

DETD(263)

Invasin.sub.718-732 linked to: HBs T.sub.h :GG:LHRH generates peptide 32, to MV.sub.F1 T.sub.h :GG:LHRH generates peptide 33, to PT.sub.2 T.sub.h :GG:LHRH generates peptide 34, to TT.sub.1 T.sub.h :GG:LHRH generates peptide 35, to TT.sub.4 T.sub.h :GG:LHRH generates peptide 36, and to TT.sub.5 T.sub.h :GG:LHRH generates peptide 37. Experiments designed to evaluate the efficacy of peptides 32-37, alone and in combination, are conducted in accordance with this and Example 13.

(2 of 9)

US PAT NO: 5,747,293 [IMAGE AVAILABLE] L4: 4 of 9

ABSTRACT:
The invention is related to **antibodies**, particularly **monoclonal antibodies**, which recognize particularly epitopes of the **intimin** protein of enteropathogenic E. coli and enterohemorrhagic E. coli, methods of detecting such E. coli by use of these **antibodies**, and kits containing these **antibodies** for diagnosis.

SUMMARY:

BSUM(1)

The present invention relates to **antibodies**, particularly **monoclonal antibodies** which recognise particular epitopes of the **intimin** protein of enteropathogenic E. coli and enterohemorrhagic E. coli, their use in the detection of such enterophathogenic E. coli, and kits containing such **antibody** for such use.

SUMMARY:

BSUM(10)

We have now generated further fusion proteins in addition to MEP-Int.sub.EPEC280 (amino acids 660-939). These fusion proteins include different amino acid sequences derived from the 280 binding domain.

MBP-Int.sub.EPEC150 (amino acids 790-939) was found to be the smallest fusion protein that had the ability to mediate cell binding. In addition the cysteine residue at position 937 of Int seems to be required for binding activity, as substitution with serine results in a loss in biological activity. Using the MBP expression system we have obtained high yields of MBPInt.sub.EPEC280, MBP-Int.sub.EHEC275, MBP-Int.sub.CF280 and MBP-Inv.sub.YP280 fusion proteins. These proteins can be used to immunise mice in order to generate specific **monoclonal antibodies**. We have now identified **monoclonal antibodies** which specifically recognise the antigen MBP-Int.sub.EPEC280 and which do not recognise MBP, MBP-Int.sub.EHEC275, MBP-Int.sub.CF280 or MBP-Inv.sub.YP280. These **antibodies** are therefore specific for the 280 amino acid domain of Int.sub.EPEC and will therefore be useful in both the detection and/or treatment of EPEC infection.

SUMMARY:

BSUM(11)

Similarly, MBP-Int.sub.EHEC275 can be used to generate monclonal **antibodies** which recognise MBP-Int.sub.EHEC275 but not MBP-Int.sub.EPEC280.

SUMMARY:

BSUM(12)

Thus, in a first aspect, the present invention provides an **antibody** which recognises a region within either the carboxy-terminus 280 amino acid domain of the enteropathogenic E. coli **intimin** protein (Int.sub.EPEC280) or the enterohemorrhagic E. coli **intimin** protein (Int.sub.EPEC 280).

SUMMARY:

BSUM(13)

In the context of the present invention, "**antibody**" refers to conventional **polyclonal** and **monoclonal antibodies** and includes antigen binding portions or fragments thereof, e.g. Fab and/or fragments of Fab.

SUMMARY:

BSUM(14)

In a preferred embodiment, the **antibody** is a **monoclonal antibody**. In particularly preferred embodiments, the **antibody** is a **monoclonal antibody** which recognises a region within the following carboxy-terminus 280 amino acid sequence:

SUMMARY:

BSUM(19)

Preferably, a **monoclonal antibody** recognising Int.sub.EPEC280 does not recognise the carboxy-terminus 280 amino acid domain of the **intimin** protein from enterohemorragic E. coli (Int.sub.EHEC 275) or Citrobacter freundii biotype 4280 (Int.sub.CF 200) or the

carboxy-terminus 280 amino acid domain of the invasion protein from Yersinia pseudotuberculosis (Inv.sub.YP 200)

SUMMARY:

BSUM(20)

More preferably, the **monoclonal antibody** recognising Int.sub.EPEC280 recognises an epitope in the 660-790 amino acid region of the enterophathogenic E. coli **intimin** protein.

SUMMARY:

BSUM(21)

Hybridomas producing **monoclonal antibodies** of the invention are also included within the scope of the invention.

SUMMARY:

BSUM(22)

The properties of the **monoclonal antibodies** of the invention render them useful for detecting enterophathogenic E. coli and enterohemorrhagic E. coli. Thus, the invention also provides the use of such **monoclonal antibodies** in detecting enteropathogenic and enterohemorrhagic E. coli.

DETDESC:

DETD(44)

The repertoire of MBP-Int.sub.EPEC fusion proteins was used in an ELISA assay to identify the region within the 280 domain that was recognised by the **monoclonal antibodies**. MBP, MBP-Int.sub.EHEC275, MBP-Int.sub.CF280 and MBP-Inv.sub.YP280 were used as controls.

DETDESC:

DETD(46)

Two productive hybridomas secreting specific anti Int.sub.EPEC280 **monoclonal antibodies** were cloned. Using an ELISA based assay we found that both **monoclonal antibodies** reacted with Int.sub.EPEC280 but not with MBP, MBP-Int.sub.EHEC275, MBP-Int.sub.CF280 and MBP-Inv.sub.YP280. In order to identify the region within the 280 domain that contain the specific epitopes, the **monoclonal antibodies** were tested against a collection of different MBP-Int.sub.EPEC fusion proteins that included in addition to MBP-Int.sub.EPEC280 (amino acids 660-939) also MBP-Int.sub.EPEC150 (amino acids 790-939), MBP-Int.sub.EPEC120C (amino acids 820-939), MBP-Int.sub.EPEC120N (amino acids 790-909), MBP-Int.sub.EPEC 70 (amino acids 870-939): MBP-Int.sub.EPEC40 (amino acids 820-859) and MBP-Int.sub.EPEC280CS (as Int.sub.EPEC280, but with Cys to Ser substitution at position 937). Both **monoclonal antibodies** recognised the 280 domain acids domain of Int.sub.EPEC but failed to react with any of the other derivatives of Int.sub.EPEC. We concluded that both **monoclonal antibodies** recognised an epitope that mapped between amino acids 660-790 of Int. Preliminary results from

agglutination assays and ELISA-based tests performed with both **monoclonal antibodies**, indicated that they can detect expression of **intimin** at the surface of several serotypes of EPEC but cannot react with eaeA mutant strain of EPEC (CVD206) or with Citrobacter freundii biotype 4280.

US PAT NO: 5,702,727 [IMAGE AVAILABLE] L4: 5 of 9

SUMMARY:

BSUM(21)

Exemplary adhesion molecules include **invasin** proteins of certain pathogenic bacteria, such as Yersina, Shigella, and Salmonella. Suitable **invasins** are encoded by the ail gene families of each of these species, as described in Leong et al. (1991) Infect. Immun. 59:3424-3433, the disclosure of which is incorporated herein by reference.

SUMMARY:

BSUM(22)

In addition to **invasins**, a suitable binding moiety may be, for example, a mono- or **polyclonal antibody** or fragment thereof, an IgA **antibody** or fragment thereof, a viral capsid, a capsid of adenovirus, cholera toxin B subunit, transferrin or a fragment thereof, lactoferrin or a fragment thereof, vitamin D, EGF or a fragment thereof, pseudomonas exotoxin, diphtheria toxin, and the like.

SUMMARY:

BSUM(24)

Particularly preferred binding moieties will contain the tripeptide arginine-glycine-aspartic acid (RGD) or the pentapeptide glutamic acid-isoleucine-leucine-aspartic acid-valine (EILDV) within their binding recognition site. The RGD tripeptide is widely conserved among many adhesive proteins present in extracellular matrices as well as being part of at least most **invasin** proteins. The RGD peptide may be utilized by itself and bound to the drug carrier particle, as described in more detail hereinbelow, or it may be obtained or synthesized as part of a larger oligopeptide which is bound to the drug carrier particle. RGD peptide is commercially available from GIBCO BRL Life Technologies, Inc. (Gaithersburg, Md.) and as Peptite-2000.TM. from Telios. Additional preferred binding moieties are the peptides arginine-glycine-aspartic acid-valine (RGDV), arginine-glycine-aspartic acid-serine (RGDS), arginine-glycine-aspartic acid-phenylalanine (RGDF), and glycine-arginine-glycine-aspartic acid-threonine-proline (GRGDTP).

SUMMARY:

BSUM(25)

It will be appreciated that the binding moiety may comprise a variety of other molecular structures, including **antibodies**, lectins, nucleic acids, and other receptor ligands, and fragments thereof. Once a desired target molecule is known, such as the integrins identified above, it will be possible to prepare or synthesize other molecules which are capable of binding the target with the requisite affinity or avidity. For example,

antibodies, including both **polyclonal** and **monoclonal** **antibodies**, may be raised against the integrin or other target molecule using conventional techniques, as described in Harlow and Lane, eds., **Antibodies**: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. (1988), the disclosure of which is incorporated herein by reference.

CLAIMS:

CLMS(5)

5. A method as in claim 1, wherein the binding moiety is selected from the group consisting of an oligopeptide containing the sequence RGD, an **invasin** molecule or a fragment thereof, EGF or a fragment thereof, and an **antibody** or a fragment thereof.

US PAT NO: 5,681,736 [IMAGE AVAILABLE] L4: 6 of 9

SUMMARY:

BSUM(32)

Ipa proteins are logical vaccine candidates although their protective efficacy has not been clearly established. Ipa B and Ipa C are immunodominant proteins (Hale, et al., Infect. Immun., 50:620-629, 1985). Furthermore, the 62 kDa Ipa B protein (the **invasin** that initiates cell entry and functions in the lysis of the membrane-bound phagocytic vacuole) (High, et al., EMBO J., 11:1991-1999, 1992) is highly conserved among Shigella species. The prolonged illness observed in malnourished children who have no significant mucosal **antibody** to Shigella Ipa suggests that the presence of mucosal **antibody** to Ipa may limit the spread and severity of infection.

US PAT NO: 5,679,564 [IMAGE AVAILABLE] L4: 7 of 9

SUMMARY:

BSUM(32)

Ipa proteins are logical vaccine candidates although their protective efficacy has not been clearly established. Ipa B and Ipa C are immunodominant proteins (Hale, et al., Infect. Immun., 50:620-629, 1985). Furthermore, the 62 kDa Ipa B protein (the **invasin** that initiates cell entry and functions in the lysis of the membrane-bound phagocytic vacuole) (High, et al., EMBO J., 11:1991-1999, 1992) is highly conserved among Shigella species. The prolonged illness observed in malnourished children who have no significant mucosal **antibody** to Shigella Ipa suggests that the presence of mucosal **antibody** to Ipa may limit the spread and severity of infection.

US PAT NO: 5,620,708 [IMAGE AVAILABLE] L4: 8 of 9

SUMMARY:

BSUM(21)

Exemplary adhesion molecules include **invasin** proteins of certain pathogenic bacteria, such as Yersina, Shigella, and Salmonella. Suitable **invasins** are encoded by the ail gene families of each of these

species, as described in Leong et al. (1991) Infect. Immun. 59:3424-3433, the disclosure of which is incorporated herein by reference.

SUMMARY:

BSUM(22)

In addition to **invasins**, a suitable binding moiety may be, for example, a mono- or **polyclonal antibody** or fragment thereof, an IgA **antibody** or fragment thereof, a viral capsid, a capsid of adenovirus, cholera toxin B subunit, transferrin or a fragment thereof, lactoferrin or a fragment thereof, vitamin D, EGF or a fragment thereof, pseudomonas exotoxin, diphtheria toxin, and the like.

SUMMARY:

BSUM(24)

Particularly preferred binding moieties will contain the tripeptide arginine-glycine-aspartic acid (RGD) or the pentapeptide glutamic acid-isoleucine-leucine-aspartic acid-valine (EILDV) within their binding recognition site. The RGD tripeptide is widely conserved among many adhesive proteins present in extracellular matrices as well as being part of at least most **invasin** proteins. The RGD peptide may be utilized by itself and bound to the drug carrier particle, as described in more detail hereinbelow, or it may be obtained or synthesized as part of a larger oligopeptide which is bound to the drug carrier particle. RGD peptide is commercially available from GIBCO BRL Life Technologies, Inc. (Gaithersburg, Md.) and as Peptite-2000.TM. from Telios. Additional preferred binding moieties are the peptides arginine-glycine-aspartic acid-valine (RGDV), arginine-glycine-aspartic acid-serine (RGDS), arginine-glycine-aspartic acid-phenylalanine (RGDF), and glycine-arginine-glycine-aspartic acid-threonine-proline (GRGDTP).

SUMMARY:

BSUM(25)

It will be appreciated that the binding moiety may comprise a variety of other molecular structures, including **antibodies**, lectins, nucleic acids, and other receptor ligands, and fragments thereof. Once a desired target molecule is known, such as the integrins identified above, it will be possible to prepare or synthesize other molecules which are capable of binding the target with the requisite affinity or avidity. For example, **antibodies**, including both **polyclonal** and **monoclonal antibodies**, may be raised against the integrin or other target molecule using conventional techniques, as described in Harlow and Lane, eds., **Antibodies**: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. (1988), the disclosure of which is incorporated herein by reference.

CLAIMS:

CLMS(4)

4. A composition comprising a matrix carrier particle composed of a material which resists proteolytic degradation and which is sufficiently small to be absorbed by a mammalian enterocyte, attached to a binding moiety selected from the group consisting of oligopeptides containing the

Ipa B Shigella (invasin)

sequence RGD, **invasin** molecules or fragments thereof, EGF or fragments thereof, and **antibodies** or fragments thereof, which bind specifically to a target molecule present on the surface of a mammalian enterocyte, the target molecule being an endocytosis- or phagocytosis-promoting receptor.

CLAIMS:

CLMS(10)

10. A composition as in claim 1, wherein the binding moiety comprises an **invasin** molecule or a fragment thereof.

CLAIMS:

CLMS(12)

12. A composition as in claim 1, wherein the binding moiety comprises an **antibody** or a fragment thereof.

CLAIMS:

CLMS(30)

30. A composition as in claim 19, wherein the binding moiety comprises an **invasin** molecule or a fragment thereof.

CLAIMS:

CLMS(32)

32. A composition as in claim 19, wherein the binding moiety comprises an **antibody** or a fragment thereof.

US PAT NO: 5,310,654 [IMAGE AVAILABLE] L4: 9 of 9

SUMMARY:

BSUM(20)

Constructs may then be prepared which may be used for introducing invasive capability into an appropriate unicellular microorganism host. Depending upon the purpose for invasiveness, a wide variety of bacterial or eukaryotic microorganism hosts may be employed. The subject method provides for introduction of DNA capability into a mammalian cell where the unicellular microorganism is employed as the vehicle for introduction of the DNA capability into a mammalian cell. For example, a shuttle vector may be provided in the invasive microorganism host which has the capability for replication in the mammalian cell as well as the unicellular microorganism, where the shuttle vector may exist as an episomal element or become integrated into the mammalian cell genome. In this manner, unicellular hosts for cloning may be used directly for the transfer of DNA into a mammalian cell host with high efficiency. Thus, a wide variety of genetic capabilities can be introduced into mammalian hosts, for example, the expression of lymphokines, hormones, enzymes, surface membrane proteins, and the like, such as interferons, interleukins, growth factors, hydrolases, oxidoreductases, receptors, **antibodies**, histocompatability antigens, etc.

SUMMARY:

BSUM(22)

A third manner in which the invasive organism may be used is as a vehicle for the introduction of molecules, particularly macromolecules, into a mammalian cellular host, either in vitro or in vivo. For example, cytotoxic resistance provided by an enzyme could be transferred into cells or a cytotoxic agent, e.g., aminoglycosides, hybritoxins, etc., non-cytotoxic to the microorganism could be introduced into mammalian cells. Dyes or other contrast agents could be introduced into the cells for visualization of cell features. Labelled **antibodies** could be introduced into the cells to define the location of particular antigens. **Invasin** proteins may be used to introduce particles, such as colloidal particles, liposomes, slowly degrading or slow release particles, cells, or the like, where the particles may include drugs, dyes, nucleic acid, **antibodies**, or other substances which may have physiological activity. The **invasin** proteins may be bound non-diffusibly to the particles, either covalently or non-covalently. The literature has numerous examples of commercially available crosslinking agents for joining proteins to other proteins, sugars, synthetic organic polymers, both addition and condensation, and the like.

SUMMARY:

BSUM(23)

Invasin proteins may also be used to bind mammalian cells to a surface. Thus in cell cultures, cells may be reversibly bound to a surface, isolated or otherwise be manipulated. Other uses will also be apparent.

SUMMARY:

BSUM(32)

The invasive microorganisms may be used to prepare antisera for passive immunization. Thus, .gamma.-globulin could be prepared which has **antibodies** to a broad spectrum of pathogens and for strains of a particular pathogen. The .gamma.-globulin may be isolated and purified from serum by ammonium sulfate precipitation and fractionation according to known techniques. Administration to a mammalian host will generally be in amounts of 50 to 500 mg/kg of host in any physiologically acceptable carrier. Administration will usually be by injection, e.g., intravenously. The modified recipient microorganism may also be used in assays for detecting the presence of **antibodies** to the antigens foreign to the modified microorganism or the antigens themselves. They also may find use in competing with the invasive microorganisms so as to be useful for therapy.

SUMMARY:

BSUM(34)

Sequences encoding all or a portion of the **invasin** genes may be used for diagnosing pathogenicity or virulence. Two fragments of interest from the ail locus are 0.9 kbp AvaI-ClaI fragment from the plasmid pVM103 and a 1.2 kb ClaI-AvaI fragment from the same plasmid or fragments thereof of at least about 50 bp, preferably at least about 100 bp or extensions

thereof including the entire ail coding region or locus. In conjunction with the ail probes, a probe referred to as Inv-Ent may be employed, which is a 3.6 kb ClaI fragment obtained from pVM101, or 50 bp fragment thereof, preferably at least a 100 bp fragment thereof. The 3.6 kb fragment comprises most of the inv gene from Y. enterocolitica in addition to adjacent sequences. Alternatively, a probe referred to a Inv-PSTB may be employed which is a 2.4 kb ClaI-XhoI fragment obtained from pRI203. Isberg et al., Cell 50:769-778 (1987). Of particular interest is the use of ail specific sequences for detecting pathogenicity. In pathogenic strains multiple copies of sequences having homology to AIL-B are frequently observed. With the Inv probes, a size pattern is observed when the microorganisms DNA is cleaved with EcoRV. These strains may be divided into types I, II, I/II, III, IV and V by the size of fragments observed. This restriction enzyme cuts once within the Inv-Ent probe, but does not cut within the AIL-B or C probes. The various types are defined in the Experimental section where, types I and I/II were found to be pathogenic, while the remaining types were non-pathogenic, with a strong correlation.

DETDESC:

DETD(86)

The above results demonstrate that Yersinia have a common invasive gene which is homologous in a plurality of species. That this gene, inv, as well as a second gene, ail, may be readily transferred to a non-invasive microorganism host to provide for invasion by such microorganism into mammalian cells. Furthermore, DNA from the genes may be used for an in vitro assay for determining pathogenicity of Yersinia species. The invasive genes find further use in identifying strains, evaluating levels of pathogenicity and relationships between pathogenicity and **invasin** genes.

DETDESC:

DETD(87)

The mammalian cells are able to endocytose the entire microbial cell based on the presence of a particular structure encoded on the surface membrane of the microorganism. The invasive phenotype can be used for diverse purposes, such as the introduction of exogenous DNA or other molecules into mammalian host, induction of an immune response to one or a plurality of antigens associated with pathogens, so as to be useful as vaccines, for production of antiserum having a spectrum of **antibodies** to a spectrum of pathogens, and for the production of proteins which may be used to inhibit invasion of pathogens in mammalian host cells.

ds

Set	Items	Description
S1	85477	R1-R5
S2	923	E2-E20
S3	328	E3-E9
S4	42	"INTIMINS"
S5	86565	S1-S4
S6	1456	INTIMIN? OR INVASIN? OR INTIMIN(N)LIKE OR INTIMIN-LIKE?
S7	86793	S5 OR S6
S8	24031	S7 AND (MONOCLONAL? OR POLYCLONAL? OR ANTIBOD? OR ANTISER? OR IMMUNOGLOB?)
S9	6331	S8/1996:1998
S10	17700	S8 NOT S9
S11	50	TARGET - S10
S12	31721	HAFNIA? OR CITROBACTER? OR YERSINA? OR EHEC? OR EPEC?
S13	1	S11 AND S12
S14	1	TARGET - S13
S15	982193	COLI OR ESCHERICHIA?
S16	1	S11 AND S15
S17	1	TARGET - S16
S18	225	AU=NYSTROM ? AND (EHEC OR COLI)
S19	80	RD (unique items)
S20	1006	S10 AND S15
S21	972	RD (unique items)
S22	50	TARGET - S21
S23	9725	O157?
S24	23	S20 AND S23
S25	16	RD (unique items)

?t s25/9/7 11 12

25/9/7 (Item 4 from file: 73)

8934636 EMBASE No: 93238377

Antigenic cross-reactions between Escherichia coli O157, **Vibrio cholerae O1 (Inaba) and group N salmonella**

Chart H.; Cheasty T.; Georgiou T.; Rowe B.
Laboratory of Enteric Pathogens, Central Public Health Laboratory, 61 Colindale Avenue, Colindale, London NW9 5HT United Kingdom

SERODIAGNOSIS IMMUNOTHER. INFECT. DIS. (United Kingdom) , 1993, 5/2 (81-84)
CODEN: SIIDE ISSN: 0888-0786
LANGUAGES: English SUMMARY LANGUAGES: English
SUBFILES: 004; 026; 028

Lipopolysaccharide antigen- **antibody** cross-reactions between **Escherichia coli O157** and other members of the Enterobacteriaceae were examined. Using serotyping schemes established in the Laboratory of Enteric Pathogens (LEP), cross-reactions were demonstrated between E. **coli O157** , Vibrio cholerae O1- Inaba and group N (O = 30) salmonella. SDS-PAGE and immunoblotting showed that the lipopolysaccharide (LPS) of E. **coli O157** contained epitopes shared with LPS expressed by group N salmonella. These cross-reactions were also detected using sera from patients with haemolytic uraemic syndrome (HUS) caused by E. **coli O157** :H7. Rabbit **antibodies** , prepared to the LPS of V. cholerae O1-Inaba reacted with the LPSs of both E. **coli O157** and V. cholerae O1-Inaba, whilst rabbit **antisera** prepared to E. **coli O157** did not react with the LPS of V. cholerae O1-Inaba. **Antibodies** in sera from patients with HUS, which reacted with the LPS of E. **coli O157** , did not react with the LPS from V. cholerae O1-Inaba. We concluded from our study that cross-reactions between E. **coli O157** and other bacterial species are rare; and that patients infected with bacteria known to share epitopes with E. **coli O157** are unlikely to give false positive results when their sera were tested for E. **coli O157** serology.

EMTAGS:

Bacterium 0762; Diagnosis 0140; Nonhuman 0777; Article 0060
DRUG DESCRIPTORS:
*bacterial antigen
MEDICAL DESCRIPTORS:
***escherichia coli** ; *vibrio cholerae; *salmonella; *hemolytic uremic syndrome--diagnosis--di
cross reaction; nonhuman; article

CAS REGISTRY NO.: 68583-22-2

25/9/11 (Item 8 from file: 73)
DIALOG(R)File 73:EMBASE

8487472 EMBASE No: 92163681
Serological cross-reactions between Escherichia coli O157 **and other species of the genus** Escherichia
Rice E.W.; Sowers E.G.; Johnson C.H.; Dunnigan M.E.; Strockbine N.A.; Edberg S.C.
U.S. Environmental Protection Agency, Cincinnati, OH 45268 USA
J. CLIN. MICROBIOL. (USA) , 1992, 30/5 (1315-1316)
CODEN: JCMID ISSN: 0095-1137
LANGUAGES: English SUMMARY LANGUAGES: English
SUBFILES: 004
The antigenic relatedness of **Escherichia coli O157** and four sorbitol- negative species of the genus **Escherichia** was examined. Isolates of **Escherichia** hermannii, E. fergusonii, E. vulneris, and E. blattae were tested in the tube agglutination assay by using **polyclonal antisera** and in the slide agglutination assay by using latex reagents. Only four isolates (17%) of E. hermannii exhibited serological cross-reactivity.

EMTAGS:
Bacterium 0762; Immunological procedures 0102; Diagnosis 0140; Apparatus, equipment and supplies 0510; Nonhuman 0777; Controlled study 0197; Priority journal 0007; Note 0063
DRUG DESCRIPTORS:
*cross reacting antigen--endogenous compound--ec; *bacterial antigen --endogenous compound--ec
sorbitol
MEDICAL DESCRIPTORS:
***escherichia coli** ; *species difference; *cross reaction
latex agglutination test; serology; bacterium isolation; agglutination test ; tube; nonhuman; controlled study; priority journal; note

CAS REGISTRY NO.: 68583-22-2; 50-70-4

25/9/12 (Item 9 from file: 73)
DIALOG(R)File 73:EMBASE

8136806 EMBASE No: 91166077
Enzyme-linked immunosorbent assay for products of the 60-megadalton plasmid of Escherichia coli **serotype** O157:**H7**
Toth I.; Barrett T.J.; Cohen M.L.; Rumschlag H.S.; Green J.H.; Wachsmuth I.K.
Division of Bacterial and Mycotic Diseases, Center for Infectious Diseases, Centers for Disease Control, Atlanta, GA 30333 USA
J. CLIN. MICROBIOL. (USA) , 1991, 29/5 (1016-1019)
CODEN: JCMID ISSN: 0095-1137
LANGUAGES: English
SUBFILES: 004
Eighty strains of pathogenic **Escherichia coli** , representing each of the major diarrheal disease-causing groups, were examined by direct enzyme-linked immunosorbent assay (ELISA) for the presence of proteins

associated with a 60-MDa plasmid from E. **coli** serotype **O157** :H7. **Antiserum** specific for plasmid-encoded proteins was prepared by immunizing a rabbit with a wild-type E. **coli** **O157** :H7 strain (strain 7785) and absorbing the serum with a plasmid-cured derivative (strain 2-45). Use of this **antiserum** in Western immunoblot analysis detected two proteins of 82 and 92 kDa in strain 7785 but not in strain 2-45. All 16 wild-type E. **coli** **O157** :H7 strains and all 10 Shiga-like toxin (SLT)-producing E. **coli** strains of serotypes other than **O157** were ELISA positive. Thirteen of 14 enterotoxigenic and all of 24 enteroinvasive E. **coli** strains were ELISA negative, as were all of 16 E. **coli** strains isolated from healthy persons. Of 16 traditional enteropathogenic E. **coli** (EPEC) serotypes, 10 were ELISA positive, including 10 of 12 strains carrying the EPEC adherence factor gene. Absorption of the serum with an EPEC adherence factor-positive EPEC eliminated EPEC reactivity. This study demonstrates that two plasmid-mediated proteins are common to E. **coli** **O157** :H7 strains and to SLT-producing strains of other serotypes. Detection of these proteins by ELISA provides a sensitive and specific screening test for identifying SLT-producing E. **coli** of both **O157** and non- **O157** serotypes. Identification of the cross-reactive proteins found in EPEC could provide the basis for a single assay to detect both EPEC and SLT-producing E. **coli** .

EMTAGS:
Heredity 0137; Immunological procedures 0102; Etiology 0135; Therapy 0160; Prevention 0165; Genetic engineering 0108; Mammal 0738; Human 0888; Priority journal 0007; Article 0060; Bacterium 0762
DRUG DESCRIPTORS:
*bacterial protein
MEDICAL DESCRIPTORS:
*serotype; *plasmid; *enzyme linked immunosorbent assay; *diarrhea --etiology--et; ***escherichia coli** **antibody** production; immunization; immunoblotting; staining; hybridization; cross reaction; gene sequence; bacterium adherence; human; priority journal; article; polyacrylamide gel electrophoresis

CAS REGISTRY NO.: 68583-22-2
?t s25/3,kwic/16

25/3,KWIC/16 (Item 1 from file: 444)
DIALOG(R)File 444:New England Journal of Med.

00108965

Medical Progress: Bacterial And Protozoal Gastroenteritis (Review Article)

Guerrant, Richard L.; Bobak, David A.
The New England Journal of Medicine
Aug 1, 1991; 325 (5),pp 327-340
LINE COUNT: 00741 WORD COUNT: 10238

TEXT
...identified bacterial pathogens, such as Helicobacter pylori, and a still growing range of types of **Escherichia** **coli** to protozoal, algal, and fungal enteric pathogens. This overview will provide an update and a...

...relatively low, according to studies in human volunteers. An infectious dose of Vibrio cholerae, Esch. **coli** , or salmonella usually requires the ingestion of 10(sup 5) to 10(sup 8) organisms...Although there have been reports of shigella, salmonella, aeromonas, cryptosporidium, and other infections, enterotoxigenic Esch. **coli** that produces either the heat-labile toxin, the heat-stable toxin, or both is consistently... proctocolitis. Diarrhea with blood, especially without fecal leukocytes, suggests enterohemorrhagic (Shiga-like-toxin producing) Esch. **coli** **O157** or amebiasis (in which leukocytes are destroyed by the parasite). Ingestion of inadequately cooked seafood...

...of giardiasis or cryptosporidiosis. Travel to tropical areas increases the chance of having enterotoxigenic Esch. **coli** infection, as well as viral (Norwalk-like or rotaviral), parasitic (giardia, entamoeba, strongyloides, or cryptosporidium...

...or infection with Staph. aureus, B. cereus (incubation periods, <6 hr), Cl. perfringens, enterotoxigenic Esch. **coli** (ETEC), vibrio, salmonella, campylobacter, shigella, or enteroinvasive Esch. **coli** (EIEC). Consider saving Esch. **coli** samples for serotyping and testing for heat-labile toxin (LT), heat-stable toxin (ST), invasiveness...tests for enterocytozoon are not yet available, serologic surveys suggest an association of Enceph. cuniculi **antibody** with tropical areas (Ref. 132). Isolated cases have improved after treatment with pyrimethamine, metronidazole, or...

...and electrolytes (Ref. 145-147). Active immunity, as well as passive protection by breast-milk **antibody** , lactoferrin, lysozyme, **antibody** , and other factors help prevent many enteric infections (Ref. 148). Finally, the protective effect of...

...important as its species in defining it as a pathogen. For example, the versatile Esch. **coli** can be normal, predominant aerobic flora or can express a range of virulence traits from...

...watery, noninflammatory diarrhea to shigella-like invasiveness, often encoded on transferrable plasmids or phage. Esch. **coli** and some other enteric organisms can produce one or more of three types of enterotoxins...

...Cl. perfringens type A, Staph. aureus, Bacillus cereus, B. fragilis, and two recently described Esch. **coli** products cause fluid secretion in animal models or electrogenic responses in Ussing chambers (Ref. 169...

...include shiga toxin and the shiga-like toxins (or vero cytotoxins) produced by ``enterohemorrhagic'' Esch. **coli** that bind to globotriaosylceramide and cleave an adenosine from host-cell ribosomal RNA to halt...toxin in causing disease. Two-to-seven-nanometer surface fibrillae or fimbriae of enterotoxigenic Esch. **coli** (colonization-factor antigens I to V) are critical to the capacity of the organism to...

...bowel, (Ref. 139,185-187) as well as in the initial colonization of enterohemorrhagic Esch. **coli** **O157** and ``enteropathogenic'' Esch. **coli** (Ref. 188,189). Enteropathogenic Esch. **coli** also exhibits localized attaching and effacing adherence and F-actin polymerization in brush-border and cultured HEp-2 cells (Ref. 190-194). Esch. **coli** with a different aggregative adherence has been associated with persistent diarrhea in studies in India and Brazil, (Ref. 195-197) and diffusely adhering Esch. **coli** , which produces a mannose-resistant hemagglutinin (Ref. 198) may also be associated with diarrhea (Ref...

...glycolipids), cell invasion may involve attachment to transmembrane glycoproteins such as integrin bound by yersinia **invasin** (Ref. 200).Shigella and enteroinvasive Esch. **coli** plasmids encode a series of **invasin** and adhesin proteins (ipa A to D) that are regulated by chromosomal codons such as...

...upper small bowel from the action of an enterotoxin (as with cholera or enterotoxigenic Esch. **coli** diarrhea) or other process that specifically alters the absorptive function of the villus tip (e.g., agents such as enteropathogenic Esch. **coli** , cryptosporidium, giardia, rotaviruses, or Norwalk-like viruses). In contrast, inflammatory dysentery usually arises in the...
...homosexual patients or those with AIDS. Specific clues may suggest inflammatory diarrhea, yersinia, enterohemorrhagic Esch. **coli** , Ent. histolytica, vibrio, Cl. difficile, giardia, cryptosporidium, enterotoxigenic Esch. **coli** , food poisoning, or sexually transmitted or other pathogens (Fig. 1). In immunocompromised patients endoscopy and...An effective, absorbable antimicrobial agent can reduce the duration of

symptoms in shigella, enterotoxigenic Esch. **coli** , vibrio, and sometimes Camp. jejuni infections. The increasing resistance of enteric pathogens to commonly used...

...the range of available effective agents (Ref. 219). For example, 44 percent of enterotoxigenic Esch. **coli** and 79 percent of shigella isolated from U.S. troops in Saudi Arabia in 1990...

...antigen is leading to new developments in vaccines against typhoid fever, cholera, and enterotoxigenic Esch. **coli** , shigella, and other enteric infections (Ref. 187,227,228...

...travel brings the affluent into increasing contact with common enteric infections such as enterotoxigenic Esch. **coli** that plague children in tropical developing countries around the world. Similarly, institutional care in day...
...advances in our understanding of the traits of microbial virulence (such as adhesins, toxins, and **invasins**) and host responses have reached the molecular level and now open exciting biochemical, immunologic, and...

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?logoff hold

ssive protective effect of chicken egg yolk immunoglobulin against experimental enterotoxigenic Escherichia coli infection in neonatal piglets

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INFECT. IMMUN. (USA) , 1992, 60/3 (998-1007)
CODEN: INFIB ISSN: 0019-9567
LANGUAGES: English SUMMARY LANGUAGES: English
SUBFILES: 004; 026

Passive protection of neonatal piglets against fatal enteric colibacillosis was achieved with powder preparations of specific antibodies against K88, K99, and 987P fimbrial adhesins of enterotoxigenic Escherichia coli. The antibody powders were obtained by spray drying the water-soluble protein fraction of egg yolks from immunized hens after the lipid components were precipitated with an aqueous dispersion of acrylic resins (Eudragit L30D-55; Rohm pharma). The anti-K88, -K99, and -987P antibody preparations reacted specifically against the corresponding fimbrial antigens in an enzyme-linked immunosorbent assay. The orally administered antibodies protected in a dose-dependent fashion against infection with each of the three homologous strains of E. coli in passive immunization trials with a colostrum-deprived piglet model of enterotoxigenic E. coli diarrhea. Scanning electron microscopy revealed adherence of enterotoxigenic E. coli in intestinal epithelial surfaces of control piglets, whereas in treated piglets treated with high-titer **antibodies** , a resistance to bacterial adhesion was observed. An enzyme immunoassay with avidin-biotin complex demonstrated specific local **antibody** activity in target areas of the small intestines. In vitro, E. **coli** K88+, K99+, and 987P+ strains adhered equally to porcine duodenal and ileal epithelial cells but failed to do so in the presence of homologous anti-fimbrial **antibodies** . Absorption of egg yolk **antibodies** with fimbrial immunosorbent removed the anti-fimbrial **antibody** fraction and reduced significantly the protective nature of the **antibody** preparation in a passive immunization experiment, suggesting that anti-fimbrial **antibodies** were the active components.

EMTAGS:
Bacterium 0762; Infectious diseases 0310; Diagnosis 0140; Therapy 0160; Etiology 0135; Prevention 0165; Swine 0729; Mammal 0738; Digestive system 0935; Small intestine 0941; Bird 0703; Chicken 0709; Nonhuman 0777; Animal experiment 0112; Controlled study 0197; Animal tissue, cells or cell components 0105; Newborn 0013; Infant 0014; Child 0022; Oral and intragastric drug administration 0181; Priority journal 0007; Article 0060
DRUG DESCRIPTORS:
*immunoglobulin--drug dose--do; *immunoglobulin--drug therapy--dt; *immunoglobulin--endogenous compound--ec
adhesin--endogenous compound--ec
MEDICAL DESCRIPTORS:
*escherichia coli; *experimental infection--diagnosis--di; *experimental infection--drug therapy--dt; *experimental infection--etiology--et; *passive immunization
swine; bacterium adherence; egg yolk; fimbria; diarrhea--drug therapy--dt; diarrhea--etiology--et; intestine epithelium cell; duodenum; ileum; colostrum; dose response; chicken; nonhuman; animal experiment; controlled study; animal cell; newborn; oral drug administration; priority journal; article
EMCLAS DRUG CODES:
03700000000

CAS REGISTRY NO.: 68583-22-2; 9007-83-4

22/9/24 (Item 24 from file: 73)

DIALOG(R)File 73:EMBASE

8934636 EMBASE No: 93238377

Antigenic cross-reactions between Escherichia coli O157, Vibrio cholerae

O1 (Inaba) and group N salmonella
Chart H.; Cheasty T.; Georgiou T.; Rowe B.
Laboratory of Enteric Pathogens, Central Public Health Laboratory, 61 Colindale Avenue, Colindale, London NW9 5HT United Kingdom
SERODIAGNOSIS IMMUNOTHER. INFECT. DIS. (United Kingdom) , 1993, 5/2 (81-84)
CODEN: SIIDE ISSN: 0888-0786
LANGUAGES: English SUMMARY LANGUAGES: English
SUBFILES: 004; 026; 028
Lipopolysaccharide antigen-antibody cross-reactions between Escherichia coli O157 and other members of the Enterobacteriaceae were examined. Using serotyping schemes established in the Laboratory of Enteric Pathogens (LEP), cross-reactions were demonstrated between E. coli O157, Vibrio cholerae O1- Inaba and group N (O = 30) salmonella. SDS-PAGE and immunoblotting showed that the lipopolysaccharide (LPS) of E. coli O157 contained epitopes shared with LPS expressed by group N salmonella. These cross-reactions were also detected using sera from patients with haemolytic uraemic syndrome (HUS) caused by E. coli O157:H7. Rabbit **antibodies** , prepared to the LPS of V. cholerae O1-Inaba reacted with the LPSs of both E. **coli** O157 and V. cholerae O1-Inaba, whilst rabbit **antisera** prepared to E. **coli** O157 did not react with the LPS of V. cholerae O1-Inaba. **Antibodies** in sera from patients with HUS, which reacted with the LPS of E. **coli** O157, did not react with the LPS from V. cholerae O1-Inaba. We concluded from our study that cross-reactions between E. **coli** O157 and other bacterial species are rare; and that patients infected with bacteria known to share epitopes with E. **coli** O157 are unlikely to give false positive results when their sera were tested for E. **coli** O157 serology.

EMTAGS:
Bacterium 0762; Diagnosis 0140; Nonhuman 0777; Article 0060
DRUG DESCRIPTORS:
*bacterial antigen
MEDICAL DESCRIPTORS:
*escherichia coli; *vibrio cholerae; *salmonella; *hemolytic uremic syndrome--diagnosis--di
cross reaction; nonhuman; article

CAS REGISTRY NO.: 68583-22-2

22/9/42 (Item 42 from file: 73)
DIALOG(R)File 73:EMBASE

7324859 EMBASE No: 89041160
Serum antibodies to Escherichia coli serotype O157:H7 in patients with hemolytic uremic syndrome
Chart H.; Scotland S.M.; Rowe B.
Division of Enteric Pathogens, Central Public Health Laboratory, London NW9 5HT United Kingdom
J. CLIN. MICROBIOL. (USA) , 1989, 27/2 (285-290)
CODEN: JCMID ISSN: 0095-1137
LANGUAGES: English
SUBFILES: 004; 025; 026; 028; 048
Sera from 13 patients with hemolytic uremic syndrome (HUS) and 8 healthy controls subjects were examined for **antibodies** specific for bacterial antigens of **Escherichia coli** serotype O157:H7. Bacterial components, including outer membrane proteins (OMPs), lipopolysaccharide (LPS), and flagella, were reacted with sera by sodium dodecyl sulfate-polyacrylamide gel electrophoresis followed by immunoblotting and by enzyme-linked immunosorbent assay. All 13 serum samples from HUS patients contained high-titered **antibodies** of the **immunoglobulin** M class against O157 LPS and some OMPs. These same sera reacted weakly with some of the major OMPs, but not the LPS, of non-O157 strains of E. **coli** . Sera from patients did not contain **antibodies** to non-O157 LPS or H7 flagella. The possibility of using E. **coli** serotype O157 LPS in an enzyme-linked immunosorbent assay for the routine diagnostic testing of sera from HUS patients for evidence

of O157:H7 infection is discussed.

EMTAGS:
Bacterium 0762; Blood and hemopoietic system 0927; Urinary tract 0950; Enzyme 0990; Preschool child 0015; School child 0016; Adult 0018; Human 0888; Infectious diseases 0310; Immunological procedures 0102; Controlled study 0197; Clinical article 0152; Male 0041; Female 0042; Priority journal 0007
MEDICAL DESCRIPTORS:
*escherichia coli; *antibody detection; *serotype; *hemolytic uremic syndrome
outer membrane protein; flagellum; polyacrylamide gel electrophoresis; immunoblotting; bacterium lipopolysaccharide; antibody titer; enzyme linked immunosorbent assay; **immunoglobulin** m; preschool child; school child; adult

CAS REGISTRY NO.: 68583-22-2; 9007-85-6
?t s22/3/4

22/3/4 (Item 4 from file: 149)
DIALOG(R)File 149:IAC(SM)Health&Wellness DB(SM)

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Discrimination between intracellular uptake and surface adhesion of bacterial pathogens.
Isberg, Ralph R.
Science, v252, n5008, p934(5)
May 17,
1991
PUBLICATION FORMAT: Magazine/Journal ISSN: 0036-8075 LANGUAGE: English
RECORD TYPE: Fulltext TARGET AUDIENCE: Academic
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